



RESEARCH ARTICLE

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Reproductive Performance and Larval quality of Giant Freshwater Prawn Broodstock of Different water resources

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ABSTRACT

The study was conducted to compare the reproductive performance and offspring quality of adults of the giant freshwater prawn, *Macrobrachium rosenbergii* broodstock from four different sites (I) Ganga riverine wild breeders (GW) (II) Ganga riverine pond-reared (GP) breeders (III) Gandak riverine wild breeders (gp); and (IV) Gandak riverine pond-reared breeders (gw) were grown out in culture ponds near Gopeshwar College, Hathua and collected as broodstock at the end of grow-out culture period. *M. rosenbergii* females were individually stocked for 120 days in three 1500 L freshwater recirculation system tanks and fed a commercial diet. Ovarian development, moulting and spawning events were checked daily. In addition a number of egg and larval quality parameters were determined. The breeding frequency, fecundity, egg laying success rate, egg dimensions and egg hatchability were not significantly different between animals from the four different sources. However, there were significant differences in terms of offspring quality between the different broodstock sources. These results indicated that broodstock sourcing deserves proper attention in hatchery operations of *M. rosenbergii*. It furthermore proves that domesticated (pond-reared) animals are not necessarily inferior as breeders as compared to wild-sourced animals. The results may also point out the potential to selectively breed stocks with improved characteristics adapted to the local culture environment.

Key words: *Macrobrachium rosenbergii*, % Reproduction, % Larval Quality, % Broodstock.

INTRODUCTION

The giant freshwater prawn, *Macrobrachium* further expansion and development of *M. rosenbergii* (de Man) is the largest species in the genus culture was noticed by Phuong, *et al.*, (2006). According to and is most favored species for culture. Thang (1993) stated that poor performance (in terms of survival and metamorphosis rate) of larvae from wild captured parent stock remains a bottleneck at present about all the hatcheries using cultured brood stock only. According to Ling and Merican (1961) and New (2000) despite four decades of domestication little information is published concerning the effects of many generations of domestication on cultured stock (e.g. inbreeding level). There is still controversy whether it is better to use wild or pond-reared breeders and local or imported prawn breeders. Wild breeders are generally considered better, but quality may vary depending on capture techniques and transport conditions. Moreover, breeders from different origin might have different characteristics in terms of reproduction and offspring quality. A number of studies have been carried out comparing the reproductive performance and related variable of wild and pond reared broodstock of different species were reported by Rao (1991) and Hoa, *et al.*, (2009). Evaluating reproductive characteristics and offspring quality of different prawn strains could also be considered as a first step in the development of selective breeding programs. To date, there are Hatchery output nevertheless is still insufficient to meet demands both in terms of both quantity and quality. Therefore, large numbers of *M. rosenbergii* post larvae (PL) are imported from other countries.

In the present study, an experiment was conducted to compare the reproductive performance and offspring quality of *M. rosenbergii* broodstock from four different sources with the objective to determine which broodstock source is most suited for seed production under conditions prevailing in India. This knowledge may add to the development of improved hatchery seed quality of *M. rosenbergii* culture and serve as a starting point to set up a selective and better breeding program

MATERIALS AND METHODS

The adult prawns selected from different sources as (I) Ganga riverine wild breeders (GW); (II) Ganga riverine pond-reared breeders (GP); (III) Gandak riverine wild breeders (gw) and (IV) Gandak riverine pond-reared breeders (gp). The individual weight and length were somewhat similar at the start of trials. The number of initial breeders for each source was 40. These prawns were randomly selected and stocked into the three broodstock tanks with overhead biological filter tank as described by Cavalli, *et al.*, (1999). An aeration system provided aeration and added the water to pass through the filter media. The holding tank was divided into two rearing and one central compartment containing female brooders. Another backup tank system was also arranged to maintain extra animals from main sources for replacing any mortalities during trial period. Freshwater was continuously pumped from the central compartment into the biological filter and flowed back to the holding tank by gravity with daily 60% water exchange. The lower level of ammonia and nitrate were maintained with $29 \pm 1^\circ\text{C}$ and formulated diet (FAO, 2002). The larval rearing system was installed following the design of Cavalli, *et al.*, (2001) with three separate recirculation systems of 100 Litre capacities and water enters in larval tank from the bottom at a flow rate of 0.2-0.3 L per minute. The water salinity was maintained 10 to 12 ppt prepared by mixing sea water.

On day-1, 80 newly hatched (triplicate) larvae collected and stocked in the rearing tanks with stocking density of 75 larvae per Litre and water temperature belongs to $30 \pm 1^\circ\text{C}$ and kept on formulated feeding regime (FAO, 2002).

The mean weight and total length of male and female prawns were recorded with ovarian development classified according to the description by Chang and Shih (1995) and spawning events of each female were recorded. Fecundity was estimated randomly from each group of broodstock is about 50% of spawning. The egg clusters were removed 7 days after spawning and then incubated in laboratory to estimate egg hatchability and remaining 50% eggs in the berried prawns were allowed to hatching. The females were first dried with tissue paper and removed egg mass and somatic weights of female were determined as somatic index (ESI): Egg clutch-weight somatic-weight 1. The individual egg weight and the total number of egg per clutch were also calculated with weighting egg samples. Fecundity was expressed as the number of egg spawns-1 and the number of eggs g-1 female body weight. The gonadosomatic index (GSI) also calculated with gravid females. The spawning frequency and survival of the females over the 4 months rearing period was also calculated and larval success rate (%) was calculated as the ratio of successful larval production and total number of spawning. The egg dry weight was obtained by placing four samples at 70°C for 36 hour and moisture content was determined with difference between wet and dry weight.

The hatchability was determined with three egg samples incubation in 300-ml fibre glass tank containing diluted water at 8 ppt salinity provided moderate aeration. The hatching was calculated from the number of live larvae and dead eggs 24 hours after hatching. The hatching was also calculated based on the average larval fecundity (larvae g-1 female) in subgroup 2 and the average fecundity in subgroup 1, whereas fecundity as Total eggs/Body weight of the female and larval survival as Total larvae/Body weight of the female.

The larval quality is defined as the active larval mobility in the rearing tanks and feed consumption. The quality was observed and determined on the day 8 post-larvae based on development rate and survival and time taken for metamorphosis. The statistical

analysis performed for duration of inter-moulting, reproductive ability and offspring quality parameter with ANOVA test, while correlations through linear regression analysis.

RESULTS AND OBSERVATIONS

For the present study four brood stocks were compared. Average values of water temperature, pH, dissolved oxygen and ammonia were $29 \pm 1^\circ\text{C}$, 7.5 ± 0.4 , 5.1 ± 0.4 ppm and < 0.1 ppm, respectively throughout the experimental period.

The reproductive performance of *M. rosenbergii* females from the different stocks is presented in Table 1. The experimental design enabled to individually follow the reproductive cycle of *M. rosenbergii* females stock wise at least 5 moults and 3 consecutive spawns over the 120-days survey period. During that period some females of the Gandak river source moulted 7 times; while this was 6 times for the Ganga River pond-reared and Gandak pond-reared sources; and 5 times for the Ganga wild source. The breeding frequency of the females reached up to 5 times for the Ganga River pond-reared and Gandak river pond-reared sources. Over the 120 days of the experiment, the Ganga revarine pond-reared broodstock had the lowest survival of the larvae (60%), which was significantly different ($p < 0.05$) from the Ganga wild source (95%). The other two broodstock stocks estimated a similar value of 70%.

Table 1: Reproductive performance (mean \pm S.D.) of *M. rosenbergii* females from different stocks

Parameter	Broodstock			
	GW	GP	gW	gP
Weight (g)	42.5 ± 2.5	38.5 ± 3.8	40.5 ± 3.5	43.5 ± 1.8
Survival (%)	95 ± 1	60 ± 2	70 ± 2	68 ± 5
Inter-moult period (days)	31 ± 5	30 ± 3	30 ± 4	31 ± 4
Inter spawn period (days)	51 ± 3	50 ± 4	45 ± 4	48 ± 6
Egg laying success rate (%)	94 ± 6	82 ± 3	81 ± 5	80 ± 5
Fecundity (Egg ⁻¹ Female)	1820 ± 240	1245 ± 365	1152 ± 350	1124 ± 457
Gonadosomatic Index (%)	8.5 ± 2.5	7.1 ± 1.4	7.5 ± 1.5	7.2 ± 1.3
Egg clutch somatic index (%)	11.5 ± 3	10.9 ± 1.4	11.2 ± 2.5	10.9 ± 2.5

There was no significant ($p < 0.05$) difference in the average intermoult period of 36 days, recorded in Gandak revarine brood and River Ganga wild stocks, other are ranged around 30–31 days. Egg laying success rate ranging from 70 to 90% was also not significant. Within each broodstock source, there was also no significant difference neither in inter-moult and inter-spawn period nor in larval success rate (Table 2).

Table 2: Egg quality parameters (meant \pm S.D.) of *M. rosenbergii* females for different sources

Parameter	Broodstock			
	GW N=12	GP N=10	gW N=14	gP N=10
Egg met Height (hg)	85.2 ± 5.4	90.3 ± 1.6	89.2 ± 2.6	89.6 ± 4.2
Egg dry weight (hg)	40.5 ± 3.5	44.6 ± 2.8	41.2 ± 2.6	41.3 ± 1.6
Egg in vitro hatching (%)	67.5 ± 2.5	65.2 ± 5.4	65.12 ± 5.2	75.0 ± 2.1
Egg in Vivo hatching (%)	51	49	50	52

The fecundity expressed as number of eggs per gram female weight was approximately 1,100 eggs g⁻¹ female for all four broodstock sources. However, this parameter was highly variable between individual females (SD-364). The gonado-somatic index (GSI) of broodstock that was sampled at stage V of ovary development showed no differences between the broodstock sources and ranged from 6.9 to 8.1%. While, the egg clutch somatic index (ESI) ranged from 9.3 to 10.2%.

The individual wet weight and dry weight of the eggs were similar between the treatments, ranging from 84.2-90.3 g egg⁻¹ and 40.5-43.6 g egg⁻¹ respectively. Also the moisture content of the eggs was the same between the treatments, ranging from 54 to 56%. The egg incubation periods in laboratory were also similarly between the broodstock sources. It took approximately 21 days at a water temperature of 30±1°C for the eggs to hatch. The egg hatching in the laboratory of the treatments ranged from 62 to 75%, which was higher than the field *hatching*, which ranged from 49 to 54%.

The present study shows there was no significant difference in the reproductive parameters between the different brood stocks. Also the characteristics of the eggs originating from the different broodstock sources were similar in terms of weight and hatching properties.

Larval Quality:

Larval performance was observed in a remaining group of (second group) of breeders that was stocked to maintain until their entire egg clutch until hatching. Each female produced an average between 20,000 and 28,000 larvae during each hatching period (Table 3). The number of newly-hatched larvae per female body weight unit was around 600 to 700 larvae g⁻¹, and was no difference between the different broodstock stocks. The dry weight of the newly-hatched larvae of the Ganga riverine wild and Gandak riverine wild sources was significantly higher than for the Gandak riverine pond-reared stock ($p < 0.05$).

Table 3: Offspring quality (mean ± S.D.) of *M. rosenbergii* females from different sources

Parameter	Broodstock			
	GW	GP	gW	gP
Larvae/hatching event (n=16)	28260±10584	21542±1254	20121±657	22506±2545
Larval fecundity (larva gr/female)	702	624	602	658
Hatched larvae dry weight (mg)	28 ± 3	26 ± 2	25 ± 1	28 ± 2
Larvae dry wt. on day 10 (mg)	90 ± 12	106 ± 16	70 ± 19	138 ± 12
Larval survival on day 10 (%)	90 ± 3	75 ± 5	63 ± 4	80 ± 2
First PL appearance (days)	16 ± 1	22 ± 2	27 ± 3	19 ± 3
Survival up to PL (%)	75 ± 6	45 ± 9	33 ± 5	60 ± 2

When the larvae were grown, there was an also significant difference in the dry weight of eight-day-old larvae. Larvae from the Gandak rivarene pond-reared stock had the highest dry weight (138 g), followed by those originating from Ganga riverine pond-reared breeders (106 g) and Ganga riverine wild breeders (90 g); while the lowest weight was observed for larvae from the Gandak riverine source (70 g). A similar trend was observed for the survival on day 10 (Table 9). The larval stage index (LSI) on day 5 and 10 showed that the development of larvae from the Ganga riverine wild and Gandak pond-reared sources was significantly faster than for larvae of the Ganga Pond reared and Gandak pond-reared sources (Table 3). On day 15, LSI was very different ($p < 0.05$) between the treatments, descending in the order Ganga riverine wild, Gandak riverine wild, Gandak riverine pond-reared and Ganga riverine pond reared.

The duration of larval rearing period beginning with zoea-1 stage to the post larval appearance varies from 16-19 days. In the wild brood stock collected from River Ganga the larval rearing period (up to PL appearance) was 16 days, while in Ganga river based ponds the rearing period extended to 19 days. In the stocks collected from Gandak river side ponds it was 22 days while in the pond reared brood stocks in the Ganga region it was 27 days. Based on the duration of the rearing period from the newly-hatched larvae to the first post larval appearance, two distinct groups could be identified. The group, larval development in the stock of Ganga wild and Gandak riverine pond-reared broodstock in the rearing period significantly shorter rearing periods (16 and 19 days, respectively) in comparison with the group containing Gandak riverine side pond reared

stock and Ganga pond-reared stocks took 22 and 27 days, respectively ($p < 0.05$). The duration of larval metamorphosis from 10% up to 90% of the Gandak riverine pond-reared source was 10 days, which was significantly longer than for larvae from the three others sources.

The survival up to post larval stage of the Ganga wild (75%) and Gandak river-reared (60%) sources was significantly higher than for the Ganga riverine pond reared (45%) and Gandak riverine pond-reared (33%) sources ($p < 0.05$).

Overall, the larval development studies revealed that the larvae originating from Ganga wild and Gandak riverine cultured ponds presented considerably better results than those from wild Ganga riverine pond-reared and Gandak riverine breeders.

DISCUSSIONS

The results of the present study show that a comparison of reproductive performance of *M. rosenbergii* of different brood stocks from four different sources was largely the same in terms of egg laying capacity and reproductive capacity. This is the first study in this region on the reproductive capacities of the different brood stocks commonly utilized in this region to study the relative performance.

In the present study fecundity around 710 eggs g⁻¹ female, and was not significantly different among treatments. In a series of nutritional experiments, Cavalli, *et al.*, (1999, 2001) explained that the fecundity values around 1,450 eggs g⁻¹ female for females with an average weight of 26.2±5.1 g. Further, they stated that the efficiency of egg production tended to decrease with increasing female size. In the present study observed that the small sized females were produce higher fecundity than the larger females' or aged females. The larger size of the females used in the current study could thus account for the lower fecundity observed. Similar work are also reported by Costa and Wanninayake (1986) and Rao (1991) who reported that in wild *M. rosenbergii* populations in Sri Lanka and India, smaller females produced a higher number of eggs per unit body weight. These authors are also explained that egg size increased with increasing body size of the spawner, resulting in fewer eggs being produced. In addition, differences in feeding practices between different studies probably also affect reproductive performance. For example in the studies of Cavalli, *et al.*, (1999) proved that the supplementation of the broodstock diet with fatty acid resulted in improved reproductive performance. In our study, a common commercial feed without any supplement was fed to the breeders.

According to Rao (1991) reported that the natural environment, *M. rosenbergii* may spawn up to 4 times or more per year and similar observations made by Ling (1969). In captive conditions, Wickins and Beard (1974) showed that one female spawned 4 times in 170 days. Cavalli, *et al.*, (2001) reported that one female performed a capacity to breed up to 5 times over 180 days. In the present study, the breeding capacity reached up to 5 times in 120 days for the Penna riverine breeders. These results indicate that pond-reared prawns may be better than wild animals in terms of breeding frequency. Wild animals grew up under natural conditions which may differ from the captive conditions and therefore they may need some time to acclimate and adapt to the new conditions. Cavalli, *et al.*, (1999) explained that optimal and stable environmental conditions and balanced and constant nutrition in culture conditions may also play a significant role in this contrast to reproductive parameters. In the current study, many indicators showed differences in terms of offspring quality between the different broodstock sources.

In general, the Gandak riverine wild and Gandak riverine pond-breeders resulted in better offspring quality than the broodstock of other source. Wild breeders are having rich amount of natural nutrition. This could have not undergone stress and may not have any abnormalities raised from nutritional condition. This may very likely be responsible for the higher breeding frequency and offspring performance observed for these wild breeders. The better performance of pond-reared animals may also be the consequence of a rapid selection for animals that are adapted to grow in captivity.

The Ganga pond-reared broodstock resulted in a lower survival and generally lowers offspring quality compared to the other domesticated sources due to less adaptation to the conditions used in this experiment. These results seem to partly contradict the results of Thanh, *et al.*, (2009) who found superior growth performance when comparing exactly the same Hawaiian *M. rosenbergii* strain with two different pond-reared strains from Vietnam origin. Differences in experimental conditions may of course account for this.

Cavalli, *et al.*, (2001) explained that egg loss is considered to be partially due to consumption by the females, to the continual sloughing off of dying eggs due to epizootic infestations and to the loose nature of the larger grey eggs, which would render them more prone to physical losses. Wickins and Beard (1974) reported that egg loss during *in vivo* incubation could amount to 31% of the eggs initially deposited in the brood chamber. In contrast, Damrongphol, *et al.*, (1991) and Cavalli, *et al.*, (2001) reported that removing the eggs from females increased their reproduction output through an increased breeding frequency. Wild brood stock of freshwater prawns *M. rosenbergii*, the levels of n-3 highly unsaturated fatty acid (HUFA) particularly 20: 5n-3 of ovary increase in the ovarian development process (Cavalli, *et al.*, 2001).

The term 'larval quality' generally refers to the physiological condition of the larvae and is related to survival and growth rates during several larval developmental stages. According to Racotta, *et al.*, (2003) reported that several variables at the broodstock management level are known or suspected to affect larval quality. In the present study, the weight of the newly-hatched larvae was different although the egg wet and dry weight was not different between the broodstock sources. When the larvae were raised to postlarvae, the differences in larval rearing performance between larvae from the Gandak riverine pond-reared sources and the Gandak wild and Gandak pond-reared sources became more and more pronounced. This could clearly be demonstrated from the results of larval dry weight, larval stage index, time of appearance of post larvae and survival to post larval stage. This confirms earlier findings that larval quality is difficult to assess and might only become apparent further down the rearing cycle (Dhert, *et al.*, 1991).

CONCLUSION

This study showed that a comparison of reproductive performance of *M. rosenbergii* of different brood stocks from four different sources was largely the same in terms of egg laying capacity and reproductive capacity. This is the first study in this region on the reproductive capacities of the different brood stocks commonly utilized in this region to study the relative performance.

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